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Leis, A., Rockel, B., Andrees, L., and Baumeister, W. (2009). Trends Biochem. Sci. *34*, 60–70.

Maurer, U.E., Sodeik, B., and Grunewald, K. (2008). Proc. Natl. Acad. Sci. USA *105*, 10559–10564.

McClelland, D.A., Aitken, J.D., Bhella, D., McNab, D., Mitchell, J., Kelly, S.M., Price, N.C., and Rixon, F.J. (2002). J. Virol. *76*, 7407–7417.

Mettenleiter, T.C., Klupp, B.G., and Granzow, H. (2009). Virus Research *143*, 222–234.

Peng, L., Ryazantsev, S., Sun, R., and Zhou, Z.H. (2010). Structure *18*, this issue, 47–58.

Zhou, Z.H., Chen, D.H., Jakana, J., Rixon, F.J., and Chiu, W. (1999). J. Virol. *73*, 3210–3218.

Molecular Machines and Targeted Molecular Dynamics: DNA in Motion

Janice D. Pata[1,2,*] and Joachim Jaeger[1,2]
[1]Wadsworth Center, New York State Department of Health, Albany, NY 12201-0509, USA
[2]Department of Biomedical Sciences, University at Albany, Albany, NY 12201-0509, USA
*Correspondence: jpata@wadsworth.org
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In this issue of *Structure*, Golosov et al. present molecular dynamics simulations that illuminate the process of DNA translocation by an A-family DNA polymerase. Several distinct phases are identified that have not been visualized through crystallographic studies.

Watson and Crick (1953), in their seminal paper describing the molecular structure of DNA, comment that "it has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." After more than fifty years of research, the DNA polymerases responsible for copying the genetic material are some of the most well characterized enzymes in all of biology. Although the polymerases are divided into several different families, they all share a common two metal-ion catalytic mechanism, and most of them are described as having fingers, palm, and thumb domains: the palm contains metal-binding catalytic residues, the thumb contacts DNA duplex, and the fingers form one side of the pocket surrounding the nascent base pair.

A wide range of crystallographic and kinetic studies has demonstrated that processive DNA synthesis involves a series of conformational changes that couples nucleotide incorporation to DNA translocation (Figure 1A). The most obvious structural change during DNA synthesis is a large domain rotation that opens and closes the fingers in each cycle. The closed fingers conformation is stabilized when the incoming nucleotide (dNTP) binds to the polymerase-DNA binary complex (Pol•DNA_n) to form a pre-insertion ternary complex (Pol•DNA_n•dNTP) (Doublie et al., 1998; Kiefer et al., 1998; Li et al., 1998).

In the Family-A polymerases, DNA translocation occurs after nucleotide addition and is coupled to the release of pyrophosphate (PPi) and the opening of the fingers domain (Johnson et al., 2003; Yin and Steitz, 2004). A highly coordinated translocation process is thought to be important for preventing frameshift mutations (Johnson et al., 2003), yet intermediate steps in the translocation pathway are not likely to be captured through X-ray crystallographic methods, since they are presumably metastable. This aspect is precisely what is characterized in the study presented by Golosov et al. (2010).

The Karplus and Beese groups applied equilibrium and restricted perturbation-targeted molecular dynamics (RP-TMD) simulations to create further structural insight into the steps that take place during the complex set of events in the polymerization cycle (Figure 1A) by the Family A (polymerase I) enzymes. The large fragment of DNA polymerase I from *Bacillus stearothermophilus* (BF) is one of the best model enzymes to study processive DNA synthesis, because of the existing crystal forms that allow multiple rounds of dNTP incorporation to occur within the lattice (Johnson et al., 2003). Both the equilibrium and targeted MD simulations use as cornerstones two high-resolution structures of closed ternary (Figure 1B) and open binary (Figure 1C) complexes of BF, which provide a matched pair of structures for the simulations. The actual starting point for the calculations is a model of the ternary product complex (Pol•DNA_{n+1}•PPi), a structure that has not been determined for any of the Family-A DNA polymerases, but is available for the homologous T7 RNA polymerase (Yin and Steitz, 2004).

The RP-TMD calculations simulate transition pathways between the two crystallographically defined binary and ternary complexes by introduction of an additional energy term that steers the system along a trajectory from the pre-translocation to the post-translocation state. The intermediate structures resulting from the RP-TMD trajectories were parametrized and analyzed by following three key events that occur during the transition: (i) rotation of the O-helix;

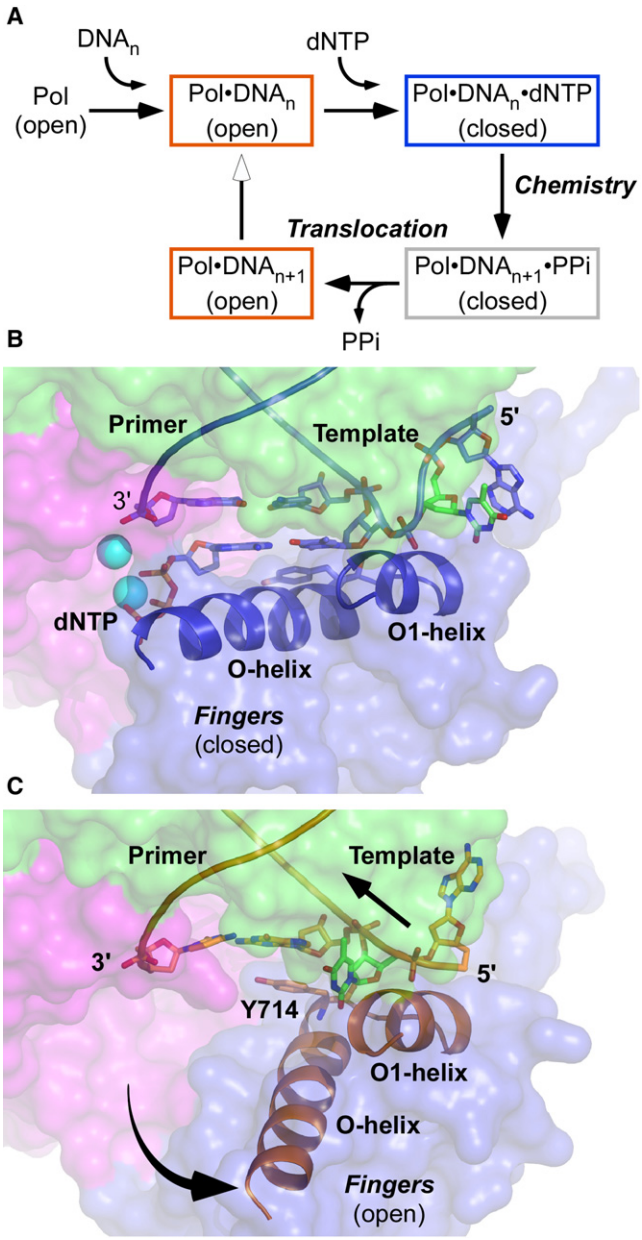

Figure 1. Conformational Changes and DNA Movement during Processive DNA Synthesis

(A) Simplified diagram of important events in the DNA polymerase catalytic cycle. Nucleotide binding stabilizes the closed polymerase structure, whereas pyrophosphate release triggers the opening of the polymerase and translocation of the substrate. The conformational state of the fingers domain at each step of the polymerase catalytic cycle is denoted in parentheses. Structural intermediates highlighted in orange and blue correspond to two crystallographically defined complexes (shown in B and C). The intermediate highlighted in gray is the complex used as the starting model for the computational study presented by Golosov et al. (2010).

(B) Closed ternary complex of BF DNA polymerase (PDB code 3EZ5). Amino acid Y714 and key bases in the DNA duplex are shown in stick representation, with the next templating base highlighted in green. The backbone of the DNA duplex and the O- and O1-helices are represented with a blue ribbon diagram. The overall outline of the DNA polymerase structure is indicated by a transparent molecular surface in domain coloring (green, thumb; magenta, palm; blue, fingers).

(C) Open binary complex of BF DNA polymerase (PDB code 3EYZ). The curved arrow shows the rotation (by ~50°) of the O-helix from the position in the closed ternary complex. The straight arrow shows the direction of DNA movement during translocation; the next templating base (green) is now located in a shallow pocket (the "pre-insertion site") between the O- and O1-helices (orange ribbon diagram).

(ii) the translocation of the DNA duplex, monitored as an average distance between residues in the palm and thumb domains and bases DNA duplex; and (iii) the distance of the next templating base to the pre-insertion pocket, between the O- and O1-helices (see Figures 1B and 1C). To gain further structural insights in the role of the pyrophosphate molecule in the post-chemistry, pretranslocation phase of the catalytic cycle, both equilibrium and RP-TMD simulations were performed in the presence and absence of PPi.

The first set of equilibrium simulations described by Golosov et al. (2010) show that there is little motion in either the protein or DNA, as long as the pyrophosphate remains bound to the polymerase. However, in the absence of PPi, the simulations (together with the translocation pathways observed by RP-TMD; discussed below) support the proposal (Yin and Steitz, 2004) that PPi release in T7 RNAP prompts DNA translocation by facilitating the opening of the fingers domain. This result adds further confidence in the computational approaches used.

The authors then follow, in detail, the conformational changes (in both the protein and the DNA) that occur after the release of pyrophosphate. Significantly, they find that translocation proceeds in three major phases (see Figure 5A of Golosov et al. [2010]). First, opening of the fingers is largely completed prior to movement of the DNA. This can occur because the O-helix in the fingers domain bends, allowing the upper end of the helix to move while the lower end (in contact with the DNA) remains in place. Second, movement of the DNA duplex occurs as the lower end of the O-helix rotates and positions Tyr714 to stack against the base in the template strand of the terminal base pair, thus coupling rotation of the O-helix to DNA translocation. Third, movement of the next templating base into the pre-insertion site occurs after the majority of the DNA duplex translocation has already taken place. The simulations indicate that a transient movement of the O1-helix provides access for the base to enter the pre-insertion site and then prevents further template movement.

While the role of Tyr714 (or Tyr639, its equivalent in T7 RNAP polymerase) had

been predicted from comparisons of open binary and closed ternary crystal structures (Johnson et al., 2003; Yin and Steitz, 2004), the simulations reported in Golosov et al. (2010) give the first indications that glycines 711 and 715 are conserved in the A-family DNA polymerases to allow for a specific flexibility in the O- and O1-helices. The importance to translocation of a flexible O-helix is unclear, since bending of the helix during the dynamics simulation occurs before DNA movement, but such flexibility could be the key for binding of the next incoming nucleotide and fingers closure, a phase that may also be studied informatively using the same computational methods.

This work highlights how computational approaches can assist in the design of site-directed mutagenesis, as well as kinetic, crystallographic, and single-molecule experimental approaches (Joyce, 2009) that are necessary to acquire a deep understanding of complex processes. Molecular dynamics is one of the few general methods available to model transient structural states in large molecular machines at the atomic level. As more structures that define a single reaction pathway become available, targeted or steered computational methods are likely to become increasingly important tools in the analysis and understanding of dynamic molecular machines.

REFERENCES

Doublie, S., Tabor, S., Long, A.M., Richardson, C.C., and Ellenberger, T. (1998). Nature *391*, 251–258.

Golosov, A.A., Warren, J.J., Beese, L.S., and Karplus, M. (2010). Structure *18*, this issue, 83–93.

Johnson, S.J., Taylor, J.S., and Beese, L.S. (2003). Proc. Natl. Acad. Sci. USA *100*, 3895–3900.

Joyce, C.M. (2009). Biochim. Biophys. Acta., in press. Published online August 7, 2009. 10.1016/j.bbapap.2009.07.021.

Kiefer, J.R., Mao, C., Braman, J.C., and Beese, L.S. (1998). Nature *391*, 304–307.

Li, Y., Korolev, S., and Waksman, G. (1998). EMBO J. *17*, 7514–7525.

Watson, J.D., and Crick, F.H. (1953). Nature *171*, 737–738.

Yin, Y.W., and Steitz, T.A. (2004). Cell *116*, 393–404.

Catching Pneumonia

Robert Liddington[1,*]
[1]Burnham Institute for Medical Research, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA
*Correspondence: rlidding@burnham.org
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Many Gram-positive bacteria have pili attached to their cell walls, but they are much simpler and shorter than their more familiar Gram-negative analogs. The structure of an ''adhesin'' from the tip of the pneumococcal pilus (Izoré et al., 2010) reveals intradomain insertions of eukaryotic origin that may hold the key to systemic invasion.

Many pathogenic bacteria have evolved to establish themselves in one organ or locale, to move on when conditions are appropriate, and to become systemic should the host be considered dispensable. One such pathogen is *Streptococcus pneumoniae* (sometimes called ''pneumococcus''), a major causative agent of pneumonia, bacterial meningitis, and bacteremia/sepsis. It is the primary killer of children in the developing world, and despite the availability of antibiotics, remains a serious threat to the elderly (Finn and Jenkinson, 2006). It is also one of the opportunistic organisms that hastens death, applying the coup-de-grâce as the immune system and major organs begin to fail, giving rise in the nineteenth century to its rather macabre label as ''the old man's friend.''

As the first step, bacteria must recognize a specific surface of the host target tissue. This often occurs on the mucosal surfaces of the nasal passages and upper respiratory tract, and is mediated by proteins called ''adhesins.'' Adhesins often contain several adhesive domains that recognize distinct host targets either with broad or fine specificity. Many bacteria augment this process by attaching adhesins to long appendages called either pili (singular = pilus = hair) or fimbriae (singular = fimbria = thread or fiber).

The highly versatile helical pili of Gram-negative bacteria were first described nearly a century ago; they are long and (relatively) thick, inserted into the outer membrane, and easily observable by the optical microscope. It is less well known that many Gram-positive bacteria have pili too, attached by covalent bonds to their peptidoglycan cell walls. But their organization is quite different; they are much thinner and shorter than their Gram-negative counterparts, and were first observed in *Corynebacteria* forty years ago using electron microscopy. Even earlier, in the 1930s and 1940s, the microbiologist Rebecca Lancefield isolated the protein components and showed that they were extraordinarily stable, strain-specific antigens (Lancefield, 1933). Although known well enough in the field of oral hygeine, Gram-positive

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